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ORIGINAL ARTICLE

Increased Eosinophilic Cationic Protein in Nasal Fluid in Hospitalized Wheezy Infants with RSV Infection

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ABSTRACT

Background: Respiratory syncytial virus (RSV) is a major respiratory pathogen which causes bronchiolitis with dyspnea and wheezing in children less than 2 years old. RSV bronchiolitis in infancy severe enough to cause hospitalization might be a risk factor for allergic sensitization and bronchial asthma in future. However, the pathophysiology behind this development has not been clearly characterized. To evaluate the existence of airway inflammation and characteristic of RSV bronchiolitis, we analyzed and compared the concentrations of eosinophilic cationic protein (ECP) in nasal fluid and plasma.

Methods: From 69 infants (aged <2 years) hospitalized for possible lower respiratory tract infections including RSV infection, we collected nasal fluid and plasma and determined the ECP concentrations.

Results: ECP concentrations in nasal fluid were significantly higher in patients with wheezing and/or bronchial rales than in patients without them (1733 ± 660 ng/mL *vs* 680 ± 450 ng/mL, $p = 0.018$), and those of the respiratory syncitial virus-infected group were significantly higher than those of the uninfected group ($p = 0.04$). Meanwhile, there was no significant difference in plasma ECP levels between patients with wheezing and patients without wheezing, and no significant difference between RSV-infected and other pathogen-infected patients. There were significant correlations between nasal fluid ECP concentrations and both neutrophil and eosinophil counts in the peripheral blood.

Conclusions: Nasal fluid ECP concentrations are increased in infants with lower respiratory infections including RSV infection accompanied with wheezing. ECP probably originates from neutrophils as well as eosinophils migrated into airways. The monitoring of ECP concentration in nasal fluid may be useful for evaluating leukocyte (including eosinophils and neutrophils)-mediated airway inflammation during infancy and its severity.

KEY WORDS

airway inflammation, eosinophilic cationic protein, eosinophils, neutrophils, respiratory syncitial virus

ABBREVIATIONS

ECP, eosinophilic cationic protein; RSV, respiratory syncitial virus.

INTRODUCTION

Persistent inflammation of the airway is thought to be an essential feature of bronchial asthma. It is generally argued that young infants with lower respiratory tract infection [in particular, respiratory syncytial virus (RSV) infection] may develop recurrent wheezy bronchitis leading to bronchial asthma. Airway inflammation in RSV bronchiolitis is a multicellular process in which epithelial cells, macrophages, cyto-

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toxic T cells and eosinophils are known to be involved.[1-4] Additionally, there is increasing direct evidence that neutrophil-mediated inflammation is involved in the pathogenesis of tissue destruction and the augmentation of bronchial reactivity in RSV bronchiolitis.[5-7]

So far, few studies have characterized the nature of airway inflammation in infants with RSV infection because bronchoscopic BAL fluid studies can be too invasive for children. ECP (eosinophil cationic protein) is an inflammatory protein which is thought to be produced by eosinophils. A number of studies have supported that ECP in induced sputum or nasopharyngeal secretion can be a useful marker in symptomatic children with asthma and in the evaluation of eosinophilic inflammation.[8,9] Meanwhile, it has also been reported that ECP originates from neutrophils as well as eosinophils, because neutrophils contain mRNA transcripts for ECP and eosinophil-derived neurotoxin.[10,11] In this study, we analyzed ECP concentrations in the nasal fluid and blood plasma of infants with lower respiratory tract infection and/or wheezy breathing including RSV bronchiolitis, and attempted to assess the relation between inflammation of respiratory tract and peripheral blood neutrophil and eosinophil counts.

METHODS

During the periods from September, 2005 to March, 2006 and September, 2006 to March, 2007, our hospitals admitted 69 infants (Table 1) aged less than 24 months with lower respiratory tract infections. Their lower respiratory tract infections were defined as lowered lung function (low SpO_2) and X-ray examinations. The diagnosis of RSV infection was made with the ABOTT Testpack RSV (Abott, Tokyo, Japan) and forty-one patients were positive for RSV.

ECP concentrations in plasma were measured at the time of hospital admission. ECP in the nasal fluid was also measured in some of the patients. For the collection of nasal fluid (50 μl), a catheter positioned at the tip of a syringe was inserted into the nasal cavity to aspirate nasal fluid. The diluted fluid was used for the determination of the ECP concentration. Each specimen was mixed well with 5 mL of sterile physiologic saline and centrifuged at 1500 *g* for 10 min, and the supernatants were kept at -20℃ until analysis. Nasal fluid and blood plasma ECP concentrations were quantitatively analyzed by a commercially available monoclonal antibody-based fluorometric immunoassay (Pharmacia, Uppsala, Sweden). The lower detection limit was <0.5 ng/mL. There were no infants in whom corticosteroid inhalation therapy or systemic corticosteroid therapy were used during hospitalization. The study protocol was approved by the ethics committee of National Fukuyama Medical Center and Okayama University Hospital, and informed consent was obtained from all patients and control subjects after the purpose of the study had been explained.

Table 1 Characteristics of the study group

Male : female	44 : 25
With RSV infection : without RSV infection	41 : 28
With wheezing : without wheezing	45 : 24
With predisposition : without predisposition	46 : 23

"With RSV infection" refers to patients whose nasopharyngeal mucus tested positive for infection using a rapid RSV diagnosis kit.

"With wheezing" refers to the presence of wheezing or bronchial rales detected during auscultation.

"With predisposition" satisfies one of the following conditions:

1. An infant with a family history of allergic diseases
2. An infant with any allergic disease other than respiratory disease
3. Total serum IgE is elevated (>200 U/mL)

All data are presented as means ± SD. Comparisons of conditions were performed with paired *t*-tests. A p value of <0.05 was considered significant in all cases. Simple regression analyses were performed with ECP concentrations in several studies.

RESULTS

ECP LEVELS IN PLASMA AND NASAL FLUID IN RELATION TO THE PRESENCE OF WHEEZING

Plasma ECP levels were measured in all patients (n = 69). There was no significant difference in the plasma ECP levels of patients with and without wheezing, (18.5 ± 16.1 ng/mL vs 12.6 ± 8.8 ng/mL). Meanwhile, ECP concentrations in the nasal fluid were significantly higher in patients with wheezing and/or bronchial rales than in patients without them (Fig. 1, 1733 ± 660 ng/mL *vs* 680 ± 450 ng/mL, p = 0.018).

ECP LEVELS IN PLASMA AND NASAL FLUID IN RELATION TO RSV INFECTION

There was no significant difference in plasma ECP levels between RSV-infected and other pathogen-infected patients. Meanwhile, ECP concentrations in the nasal fluid of RSV-infected patients were significantly higher than those of other pathogen-infected patients (Fig. 2, mean data was 1406 ng/mL *vs* 630 ng/mL, p = 0.04). The thirty-three of patients (80% of total RSV-infected patients, n = 41) had distinct wheezy breath, twenty-two (54%) had predisposition of allergy.

ECP LEVELS IN PLASMA AND NASAL FLUID IN RELATION TO ALLERGIC PREDISPOSITION

For both nasal fluid and blood plasma, there was no significant difference in ECP concentrations in patients with and without a predisposition to allergy (Fig. 3).

LINEAR REGRESSION ANALYSIS

For ECP concentrations in the nasal fluid, linear re-

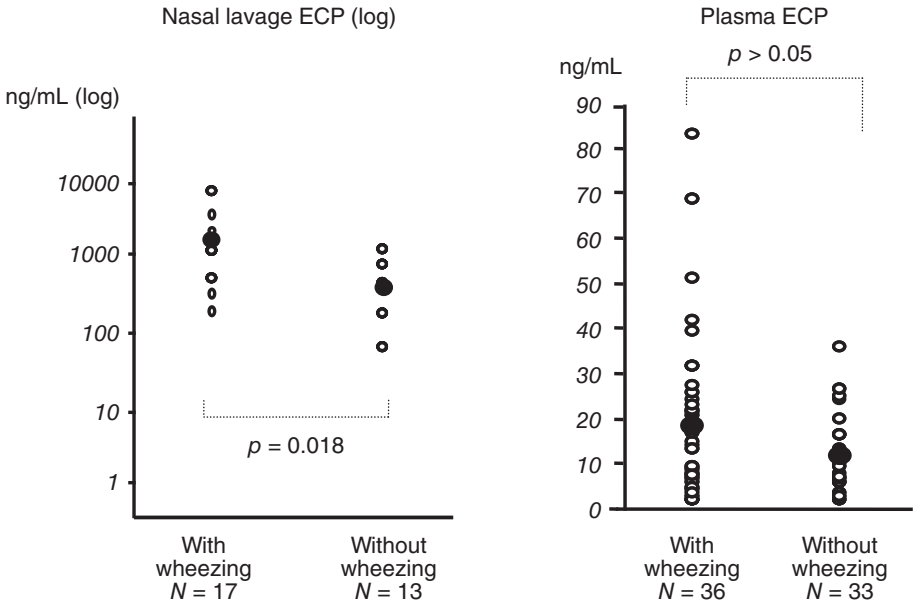

Fig. 1 ECP concentrations in nasal lavage and plasma in patients with or without wheezing. Nasal lavage ECP concentrations (log; ng/mL) were significantly elevated in patients with wheezing. Closed circle represents the mean value.

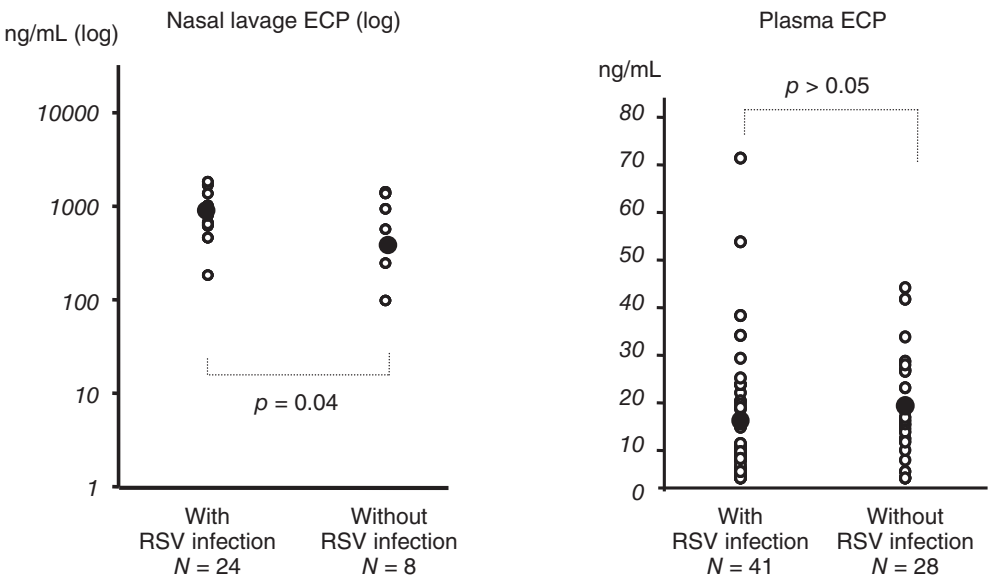

Fig. 2 ECP concentrations in nasal lavage and plasma in patients with or without RSV infection. Nasal lavage ECP concentrations (log; ng/mL) were significantly increased in patients with RSV infection. Closed circle represents the mean value.

gression analysis was performed to compare the strength of correlation with respect to wheezy bronchitis, RSV infection and peripheral blood leukocyte numbers. Statistical evidence suggested the possibility of a significant correlation between nasal fluid ECP concentrations and both neutrophil counts and eosinophil counts in peripheral blood (Table 2).

TIME-COURSE CHANGE IN ECP LEVELS IN PLASMA AND NASAL FLUID

In some patients, we collected samples twice, once on admission (infectious phase, day 0) and again at the time when respiratory symptoms diminished (recovery phase, one day before discharge; mean, 4.7 days). For both nasal fluid and blood plasma, there was no significant change in ECP concentrations during their hospitalization (Fig. 4).

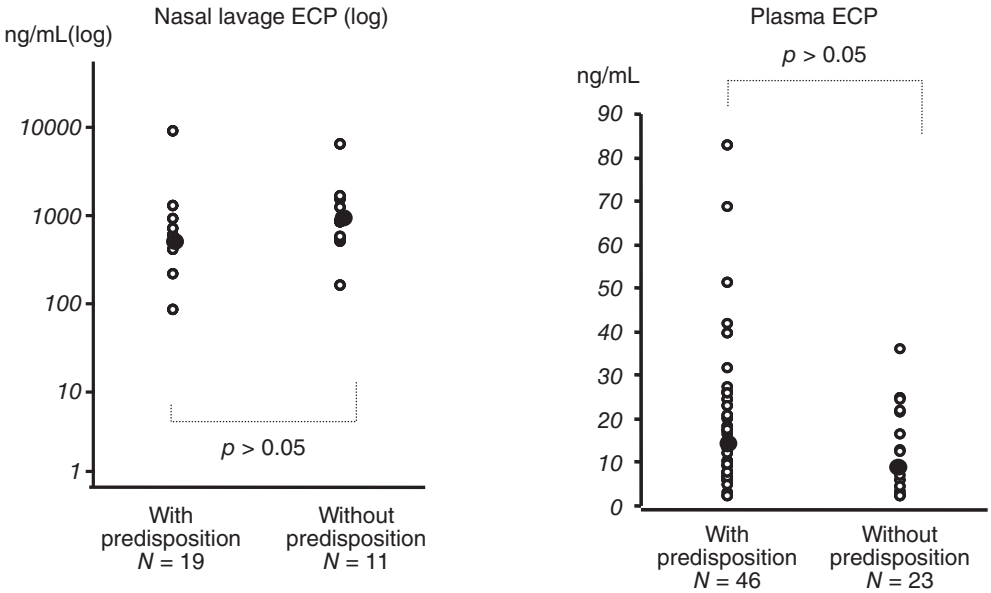

Fig. 3 ECP concentrations in nasal lavage and plasma in patients with and without allergic predisposition. No significant difference in either nasal lavage ECP or plasma ECP was found between patients with and without allergic predisposition. Closed circle represents the mean value.

Table 2 Monovariate and multivariated analysis to nasal ECP

	Infectious phase		Recovery phase	
	Monovariate (R)	Multivariate (S)	Monovariate (R)	Multivariate (S)
Allergy factor (+)	0.187		0.268*	
Wheeze (+)	0.130		0.227	
RSV infection (+)	0.099		-0.129	
Neutrophil (/μl)	0.264*		0.669**	0.570**
Eosinophil (/μl)	0.254*	0.295*	0.600**	0.446**
IgE (IU/ml)	0.035		-0.030	

RSV, respiratory syncitial virus.

Value in monovariate analysis was shown as R values of correlation.

Value in multivariated analysis was shown as S values after stepwise multiple linear regression.

*$p < 0.05$, **$p < 0.01$.

DISCUSSION

RSV is a major respiratory pathogen that causes bronchiolitis in children less than 2 years old.[12,13] The most serious manifestations of RSV infection are wheezing and respiratory distress, which often require hospitalization and artificial ventilation, Sigurs *et al*. have demonstrated that RSV bronchiolitis in infancy severe enough to cause hospitalization is a risk factor for allergic sensitization and bronchial asthma between age 3 and adolescence,[1,14,15] but the pathophysiology behind this development has not been clearly characterized. Additionally, it is also well confirmed that mild RSV infection does not increase the risk of frequent wheezing episodes and allergic sensitization in childhood.[16]

In our studies, ECP concentrations in the nasal fluid were significantly higher in hospitalized patients with wheezing or RSV infection. There was no significant difference in the plasma ECP levels of patients with and without wheezing. These results show that severe RSV and lower respiratory tract infections that contribute to the development of wheezing initiate the production of ECP in the airway, but do not influence plasma levels of ECP. ECP is an inflammatory protein produced by eosinophils, however, it is not established that only eosinophilic activation brings the symptom of severe RSV infection and wheezy breathing in the lower respiratory tract. Total eosinophil counts were not increased in all patients with

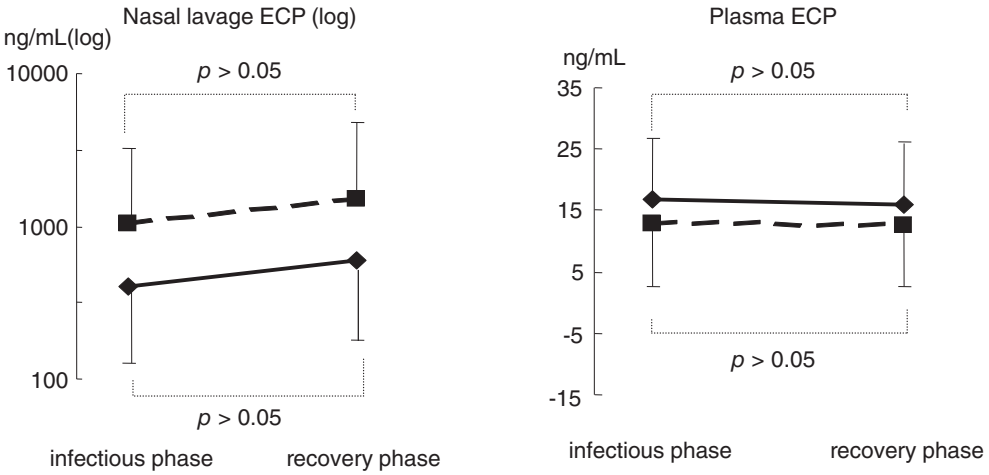

Fig. 4 Changes of ECP concentrations in nasal lavage and plasma in hospitalized patients. ECP concentrations in nasal lavage and plasma did not change with recovery regardless of RSV infection. Vertical bars show mean ± SD. ■ With RSV infection (N = 10). ◆ Without RSV infection (N = 6).

RSV infection; the eosinophil counts may not be perfectly proportional to the ECP levels in nasopharyngeal secretions from RSV-infected children as described in a previous report.[9] Some researchers have speculated that ECP originates from neutrophils as well as eosinophils, because neutrophils contain mRNA transcripts for ECP.[17] Another study suggests that neutrophils might be the stimulators of eosinophils to produce ECP.[11] In addition, our data suggest a significant correlation between nasal fluid ECP concentrations and peripheral blood leukocyte counts, not only of eosinophils also of neutrophils.

Bronchoalveolar lavage (BAL) fluid studies have indicated that in infants with RSV bronchiolitis, neutrophils are the dominant inflammatory cells, accounting for 50 to 76% of all cells in the lower airways.[5-7] During RSV infection, the inflammatory mediators (IL-8, TNF-α and granulocyte-macrophage colony-stimulating factor; GM-CSF) are present in the airways and probably originate from epithelial cells.[4-6,18] IL-8 and GM-CSF will cause an influx of neutrophils, which in turn can further contribute to airway inflammation by the release of their own chemokines and granular enzymes. Neutrophil elastase in the epithelial lining of the airways has been reported to strongly induce inflammation and destroy structures of the extracellular matrix in conjunction with RSV infections.[7,19,20] These findings strongly suggest that neutrophil-mediated inflammation also plays some role in the pathophysiology of RSV bronchiolitis.

Based on our results and previous relative findings, we proposed that measurement of ECP in nasal fluid may be useful to assessment of both eosinophilic and neutrophilic inflammation in the airway of young children. Infantile cases with severe airway inflammation induced by neutrophils and eosinophils, and especially those with RSV infection, may develop increased allergic sensitization and bronchial obstructive disease in future following airway damage. However, further longitudinal observations and quantitative studies are necessary in order to confirm this possibility.

CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

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